

# MICROBIAL COMBAT AND THE RACE TO SAVE THE FROGS: THE EFFECTS OF *BACILLUS THURINGIENSIS* SECONDARY METABOLITES ON *BATRACHOCHYTRIUM DENDROBATIDIS*

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## Background

- Batrachochytrium dendrobatidis* (*Bd*) is a fungal pathogen that infects the skin of amphibians leading to chytridiomycosis.

- Chytridiomycosis causes symptoms of appetite loss, lethargy, excessive skin shedding, and cardiac arrest resulting from ion imbalance.<sup>3</sup>

- Amphibian skin is more than just a protective barrier, it is used for ion exchange, nutrient absorption, and breathing.<sup>3</sup>

- The keratinized skin of amphibians attracts *Bd* zoospores, the zoospores burrow into the skin, develop into sporangia and release new zoospores.<sup>2,3</sup>

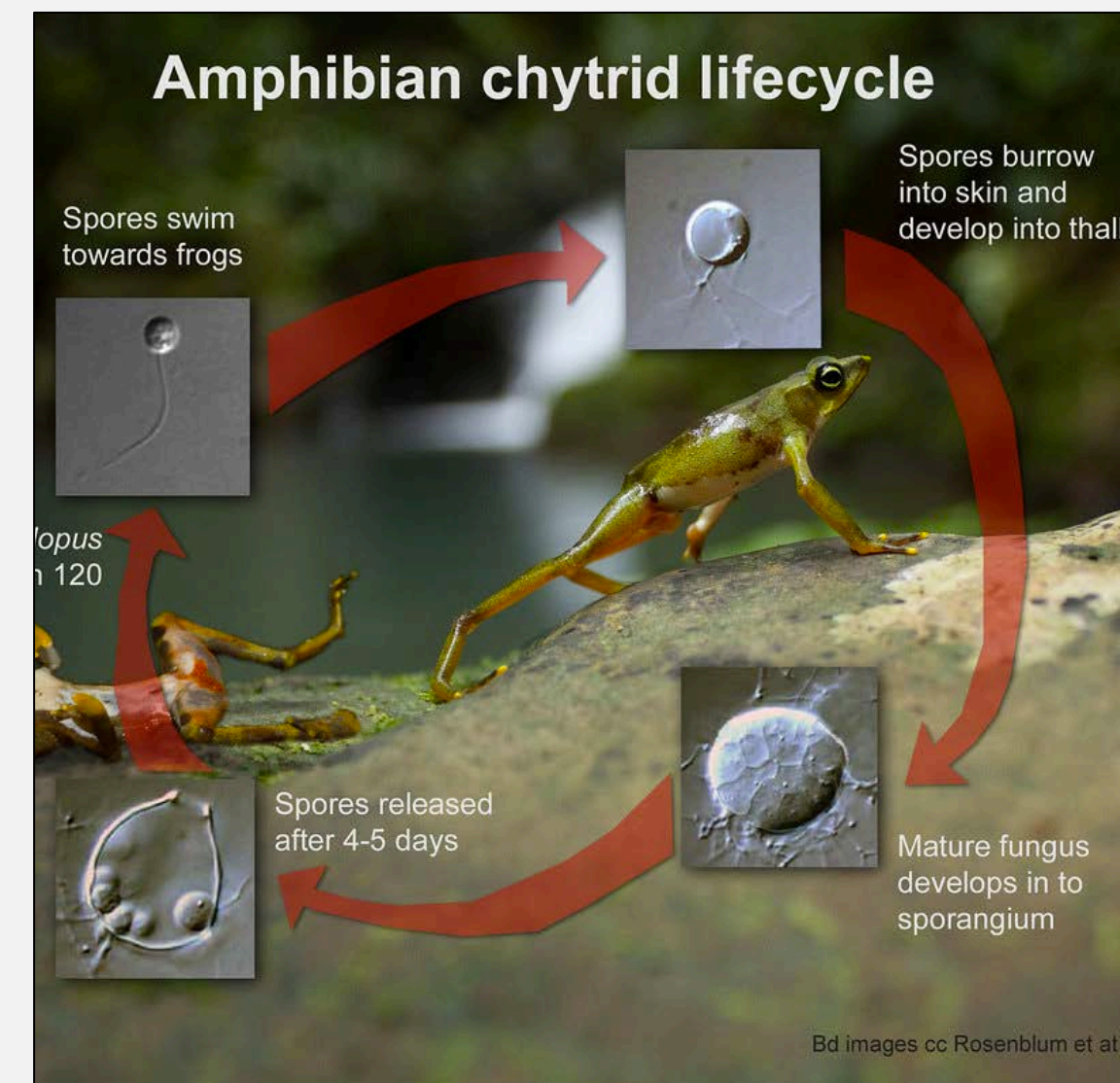


Fig. 1. The lifecycle of *Bd* and how it infects amphibians.<sup>2</sup>

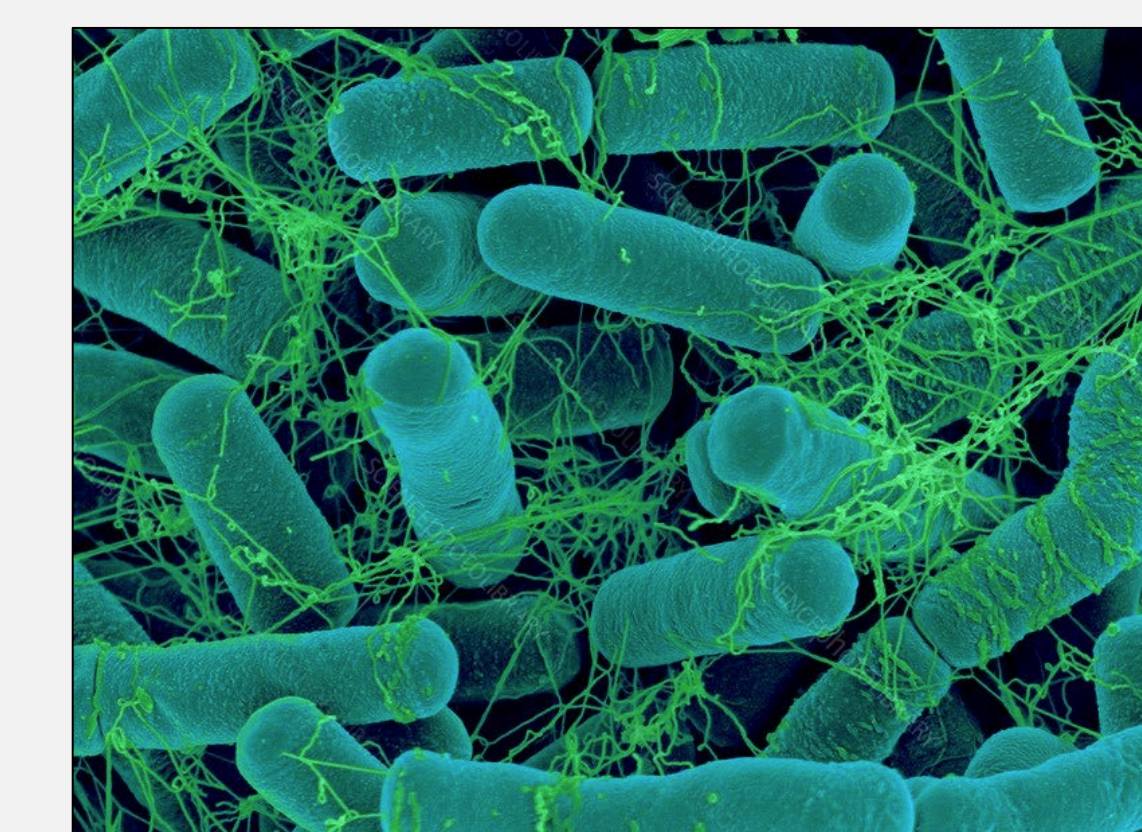


Fig. 3. *Bacillus thuringiensis* (*Bt*) soil bacteria.<sup>4</sup>

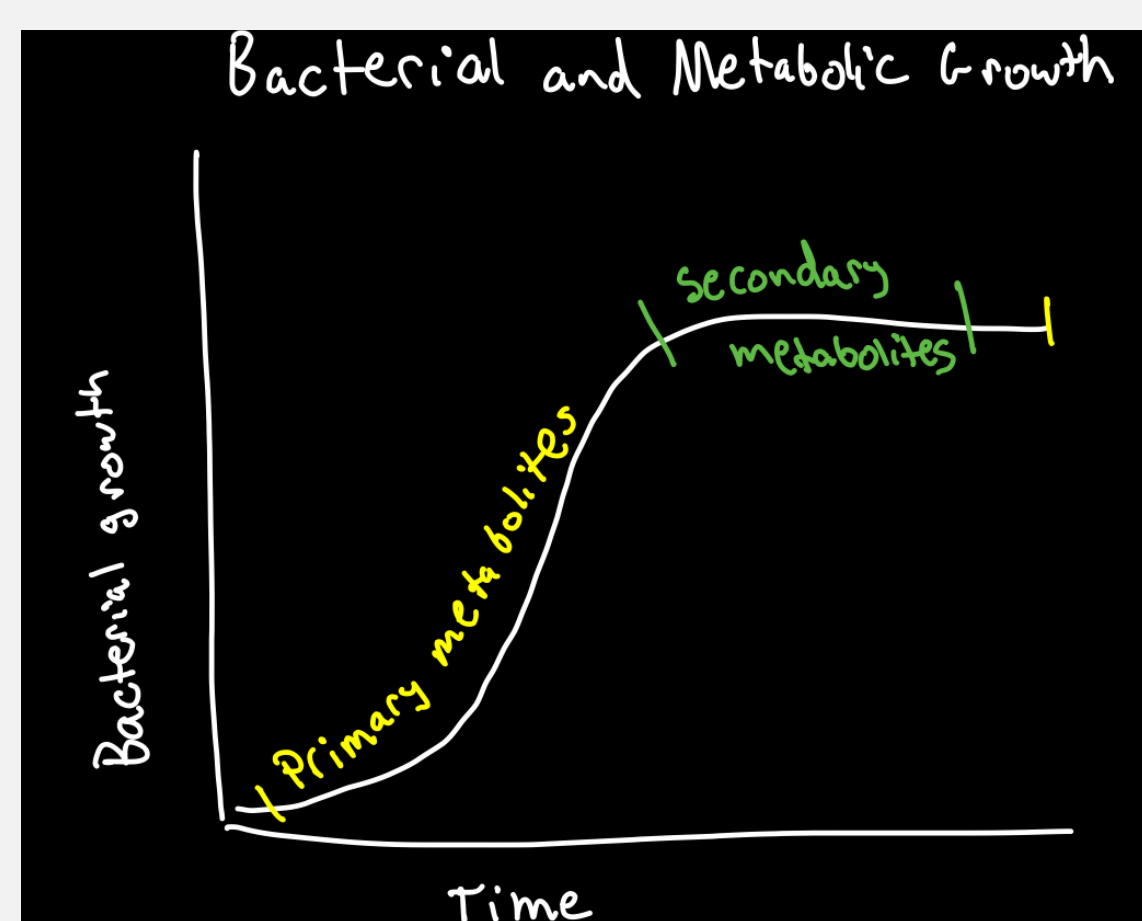


Fig. 2. Bacterial growth curve and the production of metabolites. Primary metabolites are produced during the initial growth phase, secondary metabolites are produced during the stationary phase.

- Bacillus thuringiensis* (*Bt*) is a common soil bacteria commonly used as an agricultural pesticide.

- The strain *B. thuringiensis israelensis* (*Bti*) is used to control larval growth of mosquitos and flies. *B. thuringiensis kurstaki* (*Btk*) is used to control the growth of caterpillars, particularly in corn farming.<sup>6</sup>

- Bti* and *Btk* also produce antifungal secondary metabolites.<sup>6</sup>

- Secondary metabolites are produced by many organisms, including bacteria, and some have antifungal properties.<sup>6</sup>

- Some amphibians are protected from *Bd* infection by antifungal secondary metabolites from bacteria they acquire in their environment.<sup>3</sup>

## Objectives

Assess the effects of the secondary metabolites produced by the two strains of *Bt* (*Bti* and *Btk*) on the *Bd* fungus in vitro in a liquid assay.

### Hypothesis

The presence of secondary metabolites in the liquid culture will inhibit the growth of *Bd* in vitro.

## Methods

- Bti* and *Btk* were grown separately on 1% tryptone agar plates
- Btk* and *Bti* was transferred to 1% tryptone broth, incubated at 37°C for 48 hours, and a standard growth curve was created.
- Secondary metabolites were extracted from bacterial liquid cultures and plated with the *Bd* in a 96 well plate.

- Experimental Design consisted of:

<b>Bd only (n=20)</b>
<b>Bd + 2<sup>o</sup> metabolites (n=20)</b>
<b>Heat-killed Bd + 2<sup>o</sup> metabolites (n=20)</b>

- Bt* vs. *Bd* assay was read with spectrophotometer each day for 1 week. Day 0 absorbance reading was subtracted from Day 7 reading to determine change.

- Growth enhancement calculated from:  $\frac{\text{Bd and metabolites}}{\text{Bd only}} \times 100$
- Data analyzed with two sample t-test

## Results

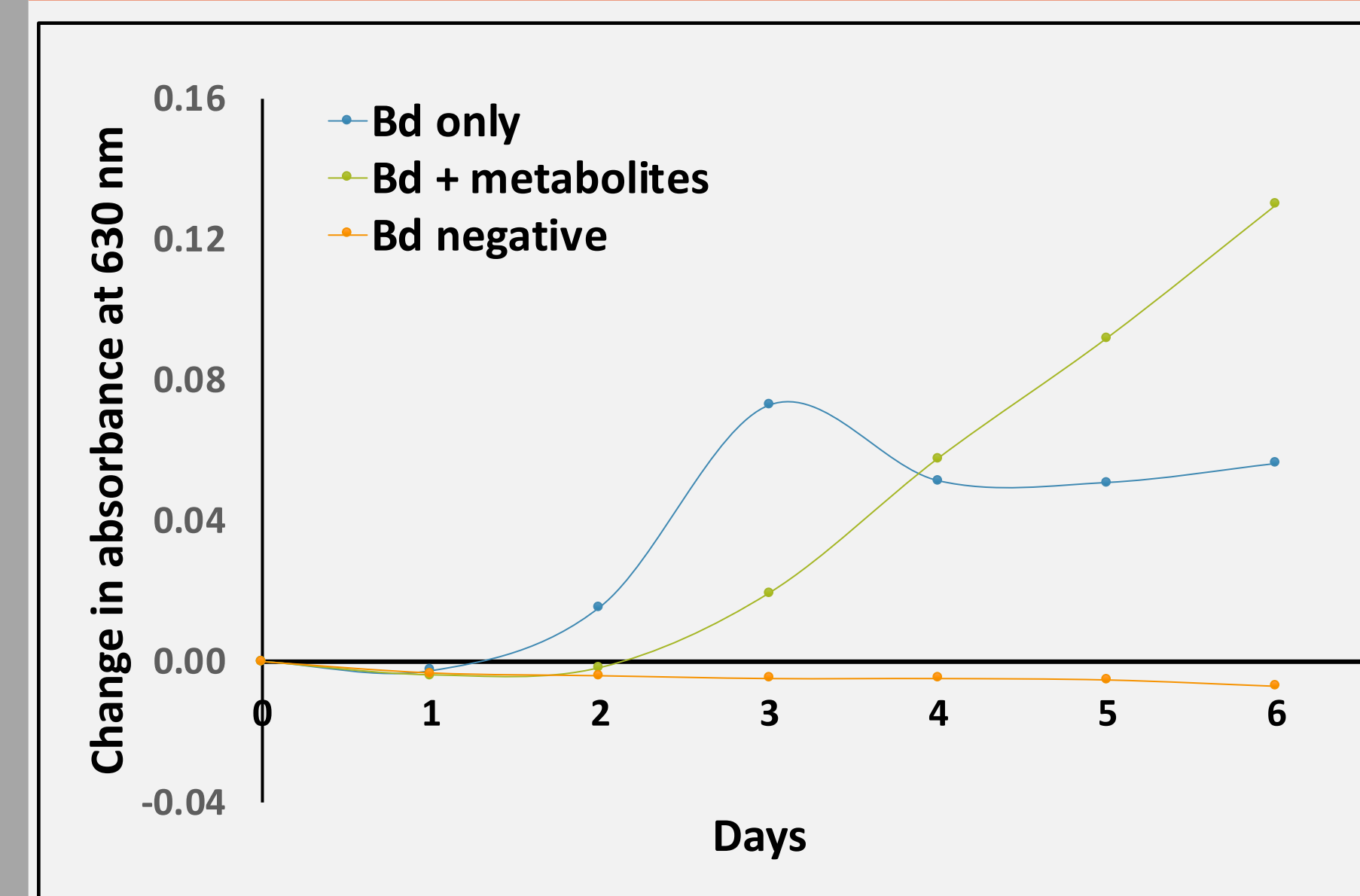


Fig. 5. *Bd* growth when exposed to *Bti* metabolites for 1 week

- Bti* metabolites significantly enhanced the growth of *Bd* by 230.5%

- $t_{18} = -10.59, p < 0.001$

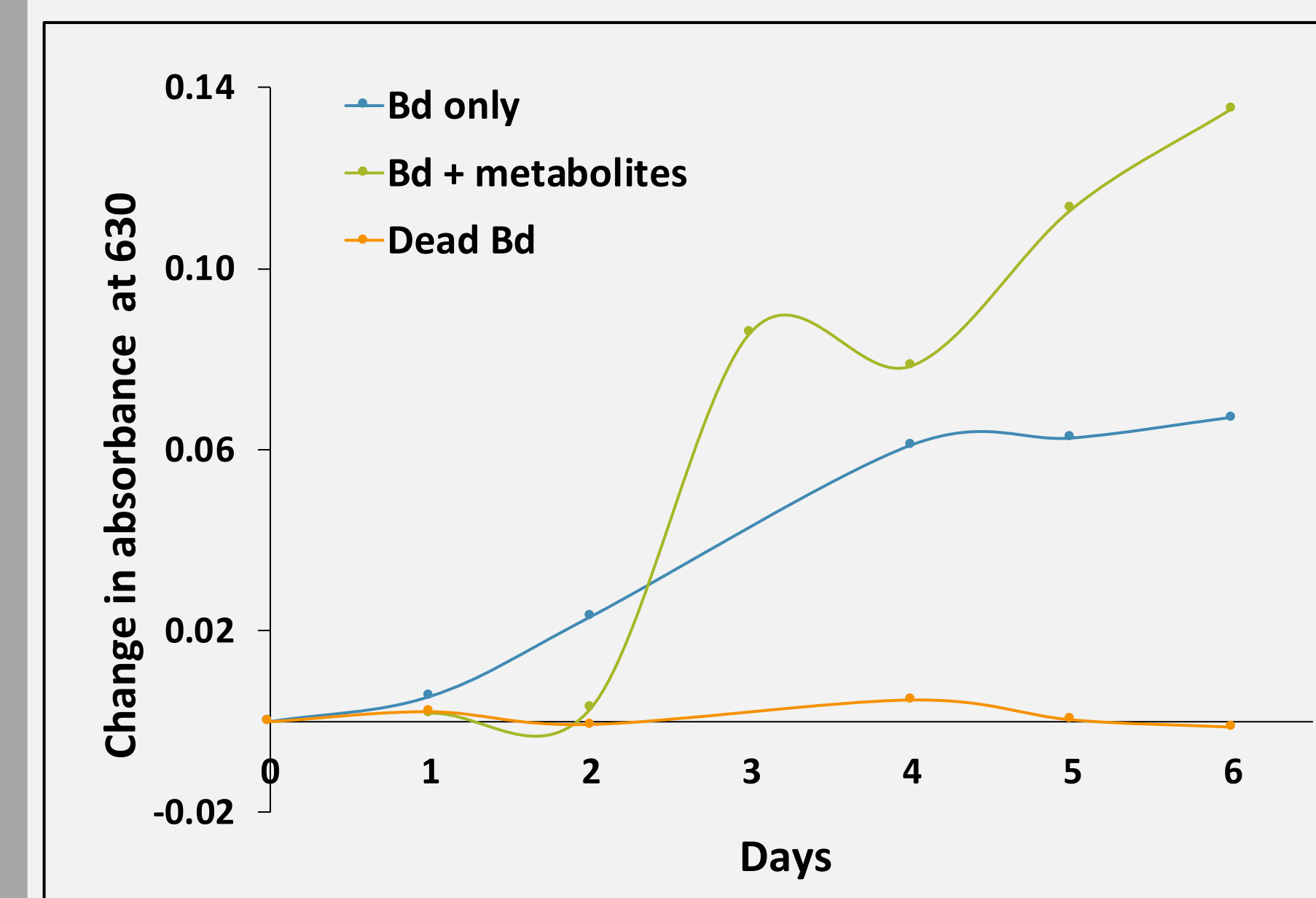


Fig. 6. *Bd* growth when exposed to *Btk* metabolites for 1 week

- Btk* metabolites significantly enhanced the growth of *Bd* by 201.5%

- $t_{18} = -21.50, p < 0.001$

## Discussion

- The growth of *Bd* was significantly enhanced by the presence of *Bacillus thuringiensis* secondary metabolites.
- Bt* is commonly used as an organic pesticide. Organic pesticides are typically thought of as better for the environment, but we do not know all their affects.<sup>5,6</sup>
- Humans release harmful chemicals into water all the time that directly effect amphibian health.<sup>3,5</sup>
- Bt*, if grown in liquid, releases secondary metabolites that may increase the growth of *Bd* in the environment if *Bt* gets into the water through crop run off.

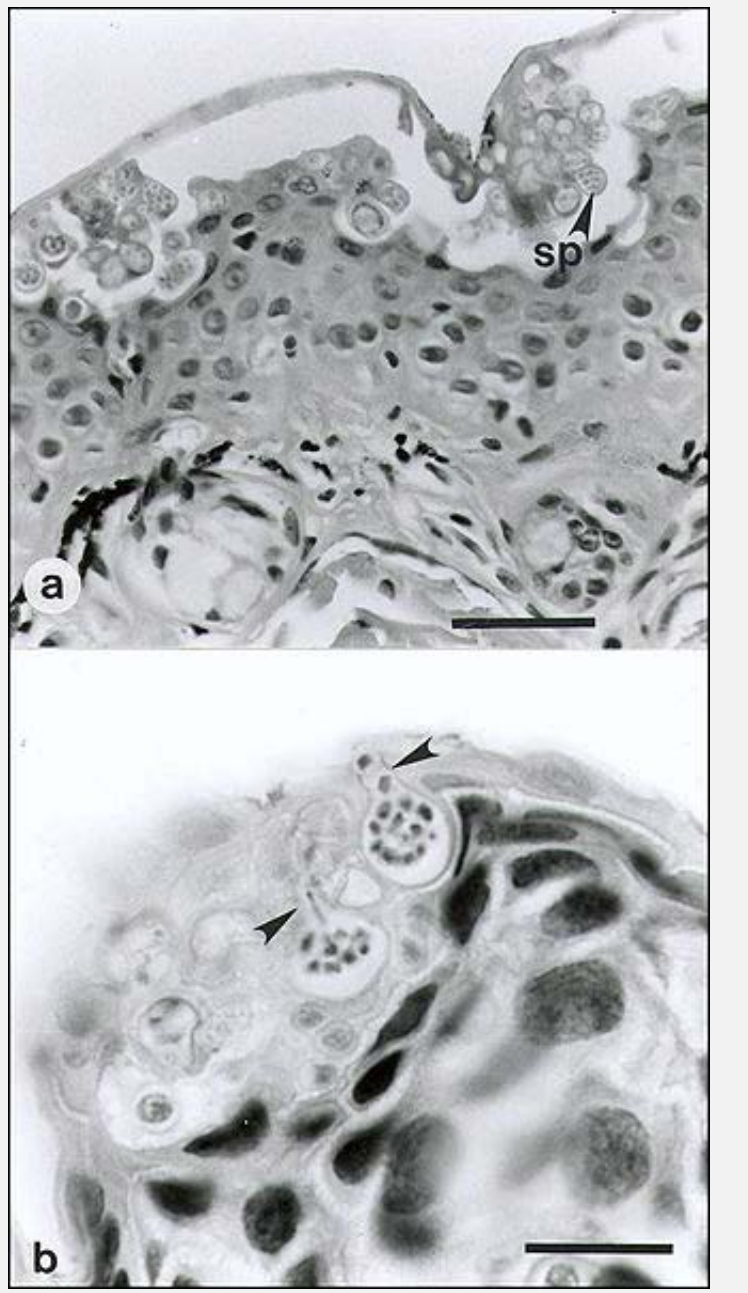
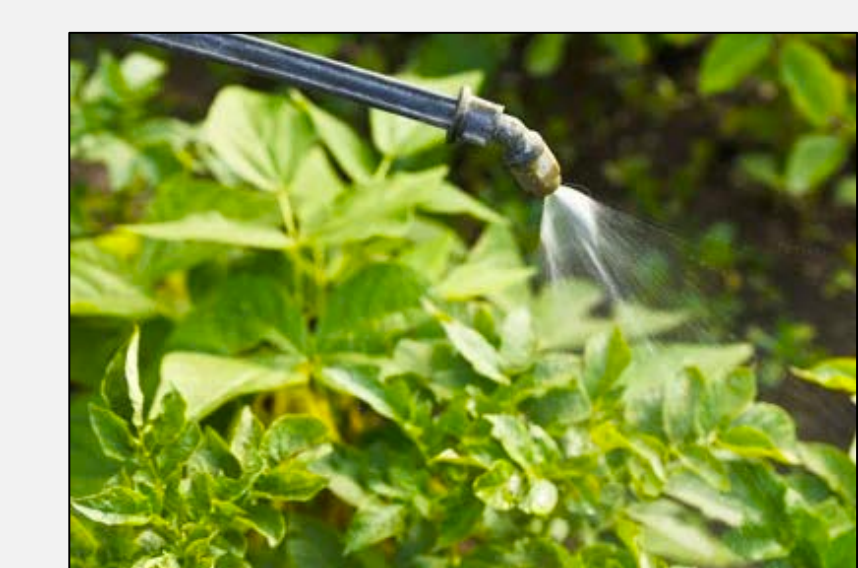
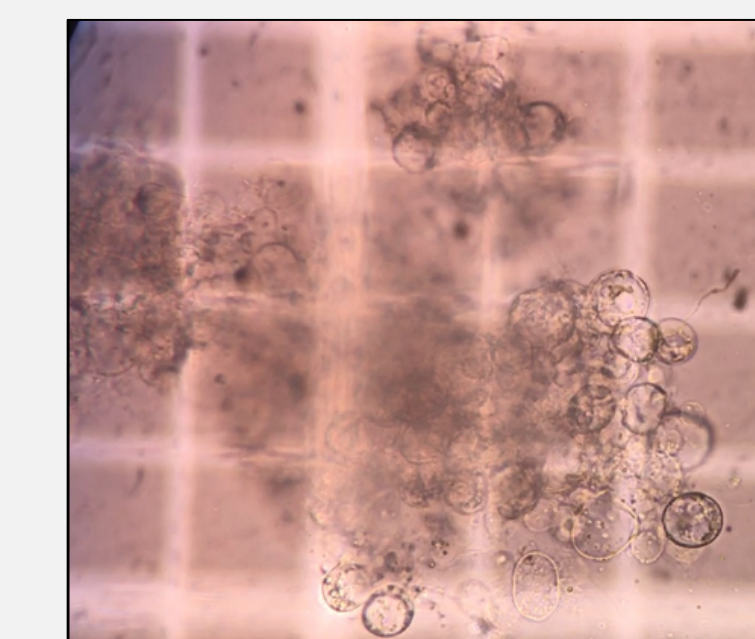


Fig. 7. *Bd* infected skin. The arrows point to zoosporangia.<sup>1</sup>



Fig. 8. Mass amphibian die-off.

Fig. 4. *Bd* zoospores & zoosporangia under the microscope (40x)



## References

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